Influences of Aortic Motion and Curvature on Vessel Expansion in Murine Experimental Aneurysms

Craig J. Goergen, Junya Azuma, Kyla N. Barr, Lars Magdefessel, Dara Y. Kallop, Alvin Gogineni, Amarjeet Grewall, Robby M. Weimer, Andrew J. Connolly, Ronald L. Dalman, Charles A. Taylor, Philip S. Tsao, Joan M. Greve

Objective—To quantitatively compare aortic curvature and motion with resulting aneurysm location, direction of expansion, and pathophysiological features in experimental abdominal aortic aneurysms (AAAs).

Methods and Results—MRI was performed at 4.7 T with the following parameters: (1) 3D acquisition for vessel geometry and (2) 2D cardiac-gated acquisition to quantify luminal motion. Male 24-week-old mice were imaged before and after AAA formation induced by angiotensin II (AngII)–filled osmotic pump implantation or infusion of elastase. AngII-induced AAAs formed near the location of maximum abdominal aortic curvature, and the leftward direction of expansion was correlated with the direction of suprarenal aortic motion. Elastase-induced AAAs formed in a region of low vessel curvature and had no repeatable direction of expansion. AngII significantly increased mean blood pressure (22.7 mm Hg, \(P<0.05\)), whereas both models showed a significant 2-fold decrease in aortic cyclic strain (\(P<0.05\)). Differences in patterns of elastin degradation and localization of fluorescent signal from protease-activated probes were also observed.

Conclusion—The direction of AngII aneurysm expansion correlated with the direction of motion, medial elastin dissection, and adventitial remodeling. Anterior infrarenal aortic motion correlated with medial elastin degradation in elastase-induced aneurysms. Results from both models suggest a relationship between aneurysm pathological features and aortic geometry and motion. (Arterioscler Thromb Vasc Biol. 2011;31:270-279.)

Key Words: aneurysms • angiotensin II • magnetic resonance imaging • elastase • near-infrared fluorescence

Abdominal aortic aneurysm (AAA) is a complex disease that leads to significant morbidity and mortality in the United States.\(^1\) AAAs are commonly defined as a 1.5-fold or larger increase in vessel diameter due to a pathological dilation.\(^2\) Diagnosis and monitoring are usually performed using noninvasive ultrasonography, but only surgical options exist to prevent continued vessel growth and reduce the risk of rupture. This “wait-and-see” approach is partially because of a lack of understanding of the mechanisms that lead to AAA development and expansion.

As a way to better study disease etiology and progression, murine AAA models have been created that mimic aspects of the human disease.\(^3,4\) In particular, 2 chemically induced murine models have become commonly used. The first model is initiated by subcutaneous systemic delivery of angiotensin II (AngII) into hyperlipidemic apolipoprotein E–deficient (apoE\(^{-/-}\)) mice, leading to suprarenal AAAs.\(^3\) The predictable formation of these aneurysms above the renal arteries in this model is of particular interest because most human AAAs are infrarenal. The rationale for the development of the second model, induced by intraluminal infusion of elastase into the murine infrarenal aorta,\(^4\) was based on the disrupted nature of elastin in human AAAs.\(^5-8\) Although both of these models produce AAAs, there are significant differences. The AngII apoE\(^{-/-}\) model is associated with atherosclerosis and often develops a hematoma,\(^9\) both of which are not commonly seen in elastase AAAs. Vessel remodeling is also different between the models because medial elastin ruptures at 1 circumferential location in AngII-infused apoE\(^{-/-}\) mice (creating a dissection between the medial and adventitial layers)\(^10\) but is degraded in a more diffuse manner in the elastase model.\(^4\) This is likely because of differences in underlying mechanisms for each model.

Despite the extensive efforts made in developing murine AAA models, the methods for quantifying disease progression remain inadequate. Most published results, using in situ or ex vivo measurements of aortic diameter, have been limited to 1 point per animal. These studies\(^6,10\) have used...
either a calibrated ocular grid to measure external AAA diameter or histomorphometry with a “shrinkage index” to estimate luminal diameter. Others have used a categorical approach in which aneurysms were assigned 1 of 4 scores based on lumen dilation, presence of thrombus, and number of bulbous expansions. Although this approach helps to classify the degree of disease progression in each animal, it does not track growth of each aneurysm over time and is not a quantitative description of AAA shape. Those who have used in vivo ultrasonography or MR to characterize AAA progression did not quantify vessel motion, curvature, or 3D expansion.

This study characterizes the influence of vessel curvature and wall motion on the location and direction of aortic expansion in experimental aneurysms, with the hope of learning more about the relationship between in vivo biomechanics and AAA pathogenesis. Newly developed MR techniques were used to measure aortic curvature, motion, and expansion at multiple points in apoE−/− mice over 28 days. To create AAAs, animals were given either a systemic infusion of AngII through an osmotic pump or an intraluminal infusion of elastase into the infrarenal aorta. After euthanasia, protease activity was estimated by the distribution of activatable fluorescent probes. Finally, histological features revealed the circumferential locations of elastin degradation, hematoma formation, and general vessel remodeling. These data suggest a correlation between biomechanics and murine AAA formation and progression.

**Methods**

A more detailed methods section is included in the supplemental material (available online at http://atvb.ahajournals.org). Experiments were performed with local institutional animal care and use committee approval. All animals used in this study were 24-week-old, male, apoE−/− mice bred on a C57BL/6 background to aid in comparisons between models.

**Surgical Procedures**

AngII-induced AAAs were created using a previously described murine model. Osmotic pumps filled with AngII were implanted in 31 mice, of which 16 survived to day 28. Of these mice, 11 developed suprarenal AAAs, defined as an expansion of at least 50% over the normal lumen diameter. Pumps filled with saline were used as a control (n=6). A detailed description of the intra-aortic elastase infusion was previously published. Briefly, the infrarenal aorta is exposed, isolated, and infused with 4.5-μM type I porcine pancreatic elastase for 5 minutes (specific activity, 5 U/mg protein at 100 mm Hg; E1250; Sigma Chemical Co, St Louis, MO) (n=28 total). Of these, 12 mice survived to day 28, all of which developed infrarenal AAAs. A control group underwent aortic infusion with heat-inactivated elastase (n=6). Buprenorphine, 0.05 mg/kg, was given to each mouse in two 50-μL subcutaneous injections before and after surgery.

**MR Vessel Imaging**

All animals underwent imaging before surgery (day 0) and on days 3, 7, 14, 21, and 28. The following MRI procedures were previously described in more detail. Briefly, all MRI was performed at 4.7 T. A 3D time-of-flight sequence, which highlights flowing blood in the aorta, was used to acquire data above and below the renal arteries. These data were used to quantify centerline shift and curvature. Maximum intensity projections of these images were used to plan orthogonal imaging planes for a cardiac-gated 2D time-of-flight sequence with 12-cine frames. These data were used to quantify cyclic strain and the direction of centroid motion.

**Image Data Analysis**

The magnitude and direction of AAA expansion were determined at the location with the greatest shift between the aneurysm centerline and the centerline of a theoretically healthy vessel (which follows the natural curvature of the aorta). The magnitude of the shift is the actual displacement, and the angle is defined relative to anterior (0°), such that a positive angle corresponds to a leftward shift and a negative angle corresponds to a rightward shift. Methods for quantifying geometric curvature from vessel centerlines were previously described. Geometric curvature (κ) was defined by the inverse of the radius of a circle as follows:

$$\kappa = \frac{1}{\text{radius}} \text{ (mm}^{-1})$$

Curvature was calculated in increments of 0.1 mm down the length of the abdominal aorta before aneurysm formation, and the location of maximum curvature was calculated in reference to the right renal artery. The lumen centroids were calculated from aortic segmentations created with a thresholding technique. The centroid of the initial timeframe was defined as the origin, and the magnitude and angle of centroid motion were calculated in reference to this location. The perimeter (P) of each segmentation was calculated by summing the distances between consecutive points around the circumference. Maximum Green-Lagrange circumferential cyclic strain was defined as follows:

$$\varepsilon_{\text{sys}} = \frac{1}{2} \left( \frac{P_{\text{sys}}}{P_{\text{dias}}} \right)^2 - 1 \times 100\%$$

where $P_{\text{sys}}$ and $P_{\text{dias}}$ are the perimeters of the vessel at systole and diastole, respectively.

**Blood Pressure and Ultrasonography**

Blood pressure was noninvasively measured with a volume pressure recording sensor and an occlusion tail cuff. Systolic, mean, and diastolic pressures were collected at each point. The same animals were imaged at day 28 with a small animal US system (Veo700). Systolic (maximum) and diastolic (minimum) diameters at the maximum AAA location were measured from M-mode tracings for ≥6 cardiac cycles per mouse.

**Histological Features**

All aortas were stained with colored dyes for circumferential orientation and then processed and embedded in paraffin. Axial sections (thickness, 5 μm) were stained with hematoxylin-eosin and elastic Van Gieson (EVG 87017) to visualize elastic lamina. Other sections were stained for smooth muscle cells using an anti–smooth muscle actin antibody (NB600–531) and macrophages using an F4/80 antibody (MCA497GA). With the AngII model, the circumferential location of adventitial hematoma and the octant corresponding to the middle of the medial elastin rupture were recorded. With the elastase model, the amount of medial elastin area was quantified by drawing regions of interest in both the anterior and posterior sections and then calculating an elastin area over the inner region of interest perimeter ratio (in micrometers).

**Near-Infrared Fluorescence**

Two activatable fluorescent probes allowed for imaging of the spatial distribution of relative protease activity (MMPSense 680 and ProSense 750). Probes were administered through a tail or jugular vein injection 24 hours before euthanasia at day 28. Fluorescent images of the excised aortas from both ventral and dorsal surfaces were acquired (FX Pro). The mean signal intensity was measured in...
the AAA, the normal thoracic aorta, and the background noise. A ratio of signal intensity was used to estimate the relative fold change as follows:

$$\text{Signal Ratio} = \frac{I_{\text{AAA}}}{I_{\text{thoracic aorta}}} / \frac{I_{\text{noise}}}{I_{\text{noise}}}$$

Each AngII AAA was then divided into 4 approximately equal quadrants to determine probe accumulation patterns within each AAA.

### Results

#### Leftward AngII AAA Expansion and Varied Elastase Expansion

AngII-induced aneurysms developed directly above the right renal artery (Figure 1A). Vessels expanded asymmetrically, forming saccular aneurysms on the left side of the aorta between the celiac and superior mesenteric arteries (supplemental Figure I). From 11 mice that developed AAAs, 1 developed an aneurysm between days 0 and 3, 1 between days 3 and 7, 8 between days 7 and 14, and 1 between days 14 and 21. At day 28, the maximum aortic diameter was located 1.09±0.57 mm above the right renal artery. In contrast, all 12 mice infused with elastase developed aneurysms at the site of the intraluminal infusion midway between the left renal artery and the aortic trifurcation (supplemental Figure II). A small initial lumen expansion was immediately seen at day 3, likely because of the mechanical damage from the pressurized infusion (Figure 1B). None of the 6 heat-inactivated control mice developed AAAs (day 0, 0.85±0.03 mm; day 3, 0.92±0.05 mm; day 28, 1.00±0.06 mm). Although vessels in both models continued to expand over 28 days, the average volume/length ratio was stable (Table 1 and Table 2). Aneurysm centerlines were calculated for both AAA models from consecutive 2D segmentations of the 3D time-of-flight acquisition (Figure 1C). Starting at day 14, the AngII AAAs shifted leftward while elastase aneurysms had no common direction of growth (Figure 1D). The elastase centerline shift magnitude increased as the vessel expanded but varied in direction.

#### Maximum Curvature and AngII AAA Formation Occur at Similar Locations

The maximum geometric curvature of the abdominal aorta (supplemental Figure III) was 0.16±0.02 mm⁻¹ and directed
leftward (supplemental Table I). This occurred slightly above the right renal artery (2.43±0.54 mm) and is located at a position where AngII-induced aneurysms form. The geometric curvature in the infrarenal aorta was anterior in direction and significantly less than the suprarenal curvature (0.09±0.02 mm⁻¹, \( P<0.05 \)). The direction of vessel curvature did not change significantly in either model before aneurysm formation (data not shown).

**Changes in Lumen Centroid Motion Differ Between Models**

Aortic centroid motion changed more substantially in the AngII model. Suprarenal aortic motion decreased in magnitude significantly between day 7 and 14 and stayed reduced through day 28 (Figure 2A). These were significant reductions of 52%, 43%, and 44% for days 14, 21, and 28, respectively, compared with day 0 (\( P<0.05 \)). The angle of centroid motion also changed at day 14, shifting roughly 30° more anterior after aneurysm formation. In the elastase model, the magnitude of centroid motion did not change dramatically (a 15% reduction at day 28 compared with day 0; \( P=0.27 \)). Yet, the angle of motion shifted rightward after surgery, with a maximum shift of 36° at day 21. This change may be because of manipulation of the peritoneum or separation of the aorta from the vena cava during the infusion procedure.

**Direction of Aortic Motion and AAA Expansion Are Similar in the AngII Model**

Mice given AngII showed a similar direction of suprarenal aortic motion at day 0 (86.9°±8.5°) and aneurysm expansion at day 28 (77.0°±24.1°). When plotted against each other, all 11 AngII AAA mice fell within the upper right quadrant (Figure 2B), corresponding to both leftward suprarenal aortic motion and leftward vessel expansion. In contrast, elastase AAA expansion was much more variable, suggesting little correlation between day 0 infrarenal motion (−3.9°±30.2°, approximately anterior) and day 28 vessel growth (17.7°±107.8°). Supplemental Table II shows the correlation factors between AAA expansion, aortic motion, and aortic curvature for both models. AngII AAAs showed a much closer correlation between all 3 than elastase aneurysms.

**Cyclic Strain Decreases in Both AAA Models**

Green-Lagrange cyclic strain waveforms for both models calculated at 12 points through the cardiac cycle are shown in Figure 3. The shape is similar for all waveforms, and a significant decrease in maximum cyclic strain is seen in both models by day 28. Mice given AngII had their maximum suprarenal strain decrease from 20.8%±4.2% at day 0 to 10.0%±2.4% at day 28 (a 52% decrease). Likewise, elastase-infused mice had their maximum infra-

### Table 1. Summary of AngII-Induced AAA Variables Over 28 Days

<table>
<thead>
<tr>
<th>Before or After AAA Day</th>
<th>No. of Mice</th>
<th>Maximum Suprarenal* Effective Diameter, mm</th>
<th>Area, mm²</th>
<th>AAA* Length, mm</th>
<th>Volume, mm³</th>
<th>Volume/Length, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before AAA</td>
<td></td>
<td>0 11</td>
<td>1.16±0.08</td>
<td>1.07±0.15</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 10</td>
<td>1.23±0.11</td>
<td>1.19±0.21</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 9</td>
<td>1.24±0.13</td>
<td>1.21±0.26</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>After AAA</td>
<td></td>
<td>14 10</td>
<td>2.61±0.48†</td>
<td>1.82±0.16†</td>
<td>2.75±0.88</td>
<td>6.15±2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 11</td>
<td>2.95±0.62†</td>
<td>1.91±0.18†</td>
<td>3.03±0.97</td>
<td>7.17±3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 11</td>
<td>3.48±1.04†</td>
<td>2.04±0.29†</td>
<td>3.25±0.98</td>
<td>8.42±3.91</td>
</tr>
</tbody>
</table>

NA indicates not applicable.

*Data are given as mean±SD.

†\( P<0.05 \) vs the vessel before surgery (day 0).

### Table 2. Summary of Elastase-Induced AAA Variables Over 28 Days

<table>
<thead>
<tr>
<th>Before or After AAA Day</th>
<th>No. of Mice</th>
<th>Maximum Infrarenal* Effective Diameter, mm</th>
<th>Area, mm²</th>
<th>AAA* Length, mm</th>
<th>Volume, mm³</th>
<th>Volume/Length, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before AAA</td>
<td></td>
<td>0 12</td>
<td>0.85±0.06</td>
<td>0.57±0.08</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 12</td>
<td>0.94±0.73</td>
<td>0.70±0.11</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 12</td>
<td>1.02±0.11</td>
<td>0.82±0.19†</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>After AAA</td>
<td></td>
<td>14 10</td>
<td>1.24±0.23†</td>
<td>1.25±0.12‡</td>
<td>4.90±0.71</td>
<td>4.36±1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 12</td>
<td>1.36±0.31†</td>
<td>1.30±0.16‡</td>
<td>4.90±1.30</td>
<td>5.02±1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 12</td>
<td>1.54±0.39†</td>
<td>1.39±0.19†</td>
<td>5.40±1.06</td>
<td>5.94±1.94</td>
</tr>
</tbody>
</table>

NA indicates not applicable.

*Data are given as mean±SD.

†\( P<0.05 \) vs the vessel before surgery (day 0).
renal strain decrease from 19.4%±2.5% at day 0 to 10.7%±1.6% at day 28 (a 45% decrease). Despite these similar reductions, the rate of change in maximum strain differed between the 2 models. AngII aneurysms had a large decrease in strain between day 7 (19.4%±7.7%) and day 14 (10.8%±1.5%), coinciding with the period of fastest vessel expansion. Elastase-induced AAAs had a more gradual decrease in strain over 28 days, with the largest decrease occurring between day 0 (19.4%±2.5%) and day 3 (15.2%±3.5%). Ultrasonographic measurements at day 28 produced similar data when compared with the MR maximum strain measurements (AngII, 8.9%±1.2%; elastase, 8.4%±2.6%; solid and dashed horizontal lines in post-AAA formation plots).

AngII Induces Hypertension and Moderately Increased Pulse Pressure

Systemic infusion of AngII over 28 days increased blood pressure (supplemental Figure IV). In mice infused with AngII, systolic blood pressure increased significantly by day 7 compared with day 0 (P<0.05), reaching a constant increase of approximately 30% (or 25 mm Hg) by day 14. Diastolic pressure showed a similar increase in days 7 through 28. Pulse pressure (calculated as systolic minus
diastolic pressure) significantly increased by approximately 9 mm Hg at days 21 and 28 ($P<0.05$).

**AngII AAA Elastin Breakdown Is Focal and Leftward**

Histological analysis confirmed vessel expansion and dissection of the aorta, with significant formation of adventitial hematomas in many AngII AAAs. Our in vivo MR images (supplemental Figure VA) were qualitatively confirmed by histological features (supplemental Figure VB) because flowing blood in the lumen and organizing hematoma both produced a signal. Often, a large hematoma formed between the medial and adventitial layers, leading to a collection of stationary blood and layered fibrin (sections 6 and 7). Other axial locations show both true and false lumens (asterisks), where blood circulated (sections 1, 2, and 3). Colored striping around the circumference (Figure 4A and 4B) showed that most AAAs experienced focal medial elastin breakdown in the left anterior region (supplemental Table III). Although the adventitial hematomas most often formed on the left side of each vessel, the right side of these AAAs appeared normal. Many of the AngII aneurysms also showed intimal thickening, smooth muscle cell migration out of the media, and prominent macrophage accumulation in the media and adventitia (supplemental Figure VI).

**Elastase AAA Elastin Breakdown Is Diffuse and Greatest in the Anterior Wall**

Elastin degradation, smooth muscle apoptosis, intimal thickening, and inflammatory infiltrate were present in the histological analysis of the elastase aneurysms. Expanded lumens are seen in both the histological sections and MRIs (supplemental Figure VII), and some AAAs had noticeable intimal hyperplasia, creating thickened vessel walls (sections 6, 7, and 8). Vessels showed significant breakdown of medial lamellar elastin, with more degradation in the anterior wall than the posterior wall (Figure 4C and 4D). Finally, immunohistochemistry revealed smooth muscle cell apoptosis in the medial layer and a mixed inflammatory infiltrate of macrophages and other mononuclear cells in the adventitia, media, and neointima (supplemental Figure VIII).

**Activatable Fluorescent Probes Colocalize to Regions of Vascular Injury**

Near-infrared fluorescent signals from matrix metalloproteinases and cathepsin-activatable fluorescent probes colocalize to regions of abdominal aortic expansion in both ventral and dorsal images (Figure 5A). Aneurysms from both AngII and elastase showed a significant 2-fold or greater increase in signal ratio compared with saline pump and heat-inactivated elastase controls ($P<0.05$, Figure 5B). A fluorescent signal may be related to vascular injury because animals infused with AngII who did not develop AAAs did not show a similar increase in the suprarenal aorta signal ratio. Furthermore, a similar pattern of activated probe accumulation was seen in most AngII AAAs (supplemental Figure IX). The left inferior quadrant showed a significant increase in normalized signal ratio compared with all other quadrants ($P<0.01$). The fluorescent signal from elastase AAAs was homogeneous and formed no repeatable pattern.
**Discussion**

The results of this study suggest that aortic curvature and pulsatile lumen expansion influence the location and direction of vessel expansion in experimental aneurysms. AngII AAAs form at the location of maximum abdominal curvature; and the direction of lumen expansion, curvature of the vessel, and shape of these saccular AAAs were all leftward in the suprarenal region. The elastase-induced aneurysms contribute as a significant negative control to the hypothesis that highly curved aortic locations with large asymmetrical centroid motion may strongly influence the direction of AAA expansion because curvature and centroid motion were much less in the infrarenal region. Thus, the finding that elastase-induced AAAs had variable directions of expansion was not surprising. The periods of largest decrease in cyclic strain coincided with the points of greatest vessel expansion in both models. The histological analysis in both models suggests a correlation between vessel breakdown and aortic motion because
regions of increased elastin degradation and the direction of lumen expansion were similar in both models. Finally, the near-infrared fluorescence images suggest that protease-activated probes colocalize to regions of vascular injury for both models.

The formation of AngII AAAs at the location of maximum abdominal curvature suggests a correlation between the amount of vessel curvature and the location of aortic dissection. Although differences in the elastin/collagen ratio or the origins of the medial cells may also play a role in AngII AAA localization, simple vessel geometry may influence the location of aneurysms as well. A correlation between the location of murine AAA formation and maximum curvature may be because of increased local strain within the left side of the suprarenal aorta or the effects of asymmetrical blood flow. Indeed, previous research in patients who experience above-knee amputation showed a propensity of aneurysms to form in the same direction as the lost limb, possibly because of flow asymmetry in the infrarenal region from the unilateral reduction of iliac artery caliber. Our results agree with these findings because flow into the superior murine right renal artery may affect the left side of the suprarenal aorta. Although these results are intriguing, caution should be taken when associating vessel curvature and asymmetrical flow with aneurysm formation because other portions of the aorta (eg, aortic arch) are more curved than the abdominal region. Yet, the effects of curvature are intriguing because others have recently observed significant lumen dilatations of the ascending aorta of AngII-infused apoE<sup>−/−</sup> mice, suggesting that vessel curvature may also play a role in thoracic aortic expansion.

The difference in AAA shape between models may be because of the underlying mechanisms that lead to vessel expansion. Our histological analysis showed aortic dissection and medial elastin breakage on the left side of the vessel in AngII AAAs, often creating true and false lumens that are associated with the formation of adventitial hematomas (supplemental Figure V). This saccular formation was seen in none of the elastase AAAs, where diffuse elastin degradation was observed around the circumference. Thus, although chemotaxis of inflammatory cells into the aortic wall occurs in both models, elastin degradation (and the mechanisms that lead to vessel expansion) is markedly different between AngII- and elastase-induced AAAs.

The pulsatile aortic motion analysis showed that strain was inversely proportional to aortic expansion. However, the rate of this decrease differed between AngII and elastase AAAs. AngII mice showed an abrupt and significant decrease temporally coinciding with AAA formation (day 14). Conversely, the elastase-infused mice had a more gradual decrease in maximum strain values, with the largest decrease between days 0 and 3. This is likely because of mechanical damage from the elastase infusion itself and not vascular remodeling because the aortic diameter had not increased significantly by day 3. The decrease in strain from days 3 to 28 may also be related to the significant increase in transmural pressure seen in the AngII model (leading to increased wall stress) or the smooth muscle cell migration and prolif-

![Figure 5. Near-infrared fluorescence signal of AAA vessels and controls. A, Example probe images (MMPSense) of the dorsal surface. The means of the regions of interest (white) were then compared with normal thoracic aorta (gray) to calculate the fold increase in signal. B, The relative fold increase in signal for both models showed significant increases in signal from the aneurysmal region.](http://atvb.ahajournals.org/)[Figure 5. Near-infrared fluorescence signal of AAA vessels and controls. A, Example probe images (MMPSense) of the dorsal surface. The means of the regions of interest (white) were then compared with normal thoracic aorta (gray) to calculate the fold increase in signal. B, The relative fold increase in signal for both models showed significant increases in signal from the aneurysmal region.](http://atvb.ahajournals.org/)
eration in the intimal layer we observed in the elastase AAAs (supplemental Figure VIII).

The enlargement we observed in elastase-induced AAAs (60% and 81% increases at days 21 and 28, respectively) did not reach the 100% threshold proposed by others. Although unexpected, the MR techniques used in this study quantified a different AAA metric. Previous research typically measured the external aortic diameter at 1 location by exposing the vessel via laparotomy. The MR technique described herein measures the in vivo internal lumen diameter of the aorta when surrounding tissue can provide restraint and does not easily measure vessel thickening.

Many proteases, including cathepsins K, L, and S and matrix metalloproteinases 2 and 9, have all been associated with human AAA disease, making them interesting targets for potential therapeutic agents. Previous work has also shown that AngII-induced murine AAAs are linked to the presence of matrix metalloproteinases. Our results suggest that proteases are not evenly distributed throughout AngII AAAs (supplemental Figure IX). In general, the inferior half of these aneurysms had increased accumulation of activated probe compared with the superior half. Furthermore, increased signal in the left inferior region of suprarenal aneurysms agrees with our histological analysis, which also showed elastin degradation and adventitial remodeling on the left side. Previous atherosclerosis research has shown that the gelatinase activity associated with extracellular matrix remodeling activates the near-infrared fluorescence probes, which then colocalize with macrophage accumulation. Others have shown that regions of thin intraluminal thrombus are associated with enhanced proteolytic activity within human AAAs. Thus, our results could indicate increased vascular remodeling or accumulation of inflammatory cells within specific regions of each aneurysm. Future work should be focused on developing in vivo near-infrared fluorescence techniques to provide insight into how active protease levels change during AAA progression.

In summary, this study shows the ability of MR angiography to evaluate aneurysm development at multiple points, highlighting differences between the 2 experimental models. The location of AngII-induced AAAs is similar to the location of maximum abdominal aortic curvature, and the leftward direction of aneurysm expansion in this model appears to be correlated with the direction of pre-AAA motion. Conversely, elastase-induced AAAs form in a region of low vessel curvature and have no repeatable direction of vessel expansion. None of these geometric or dynamic end points would be possible without the recently developed noninvasive imaging methods described herein. Future work incorporating similar in vivo imaging techniques would be useful to quantify murine AAA progression and provide the potential for improving translation because analogical imaging techniques are found in the clinic. Indeed, further research will be needed to determine if vessel curvature and pulsatile motion have an effect on human AAA expansion. Thus, this quantitative comparison between 2 commonly used murine AAA models will hopefully lead to a greater understanding of how biomechanics affect aneurysm formation and pathogenesis and help to improve future clinical treatment.

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D.Y. Kallop, A. Gogineni, Dr. Weimer, and Dr. Greve are employees of Genentech Inc.

References


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SUPPLEMENTAL MATERIAL

METHODS

All experiments were carried out with local institutional animal care and use committee approval. For all surgical and imaging procedures, animals were anesthetized with 2-3% isoflurane in 2 L/min of medical air. Animal temperature was maintained between 36° and 38° C using a heating pad during surgery and warm air during imaging. All mice used in this study were 24-week-old, male, apolipoprotein E-deficient (apoE-/-) and bred on a C57BL/6 background. Animals recovered on a heating pad and were usually fully conscious and mobile within 15 minutes. The average weight at the beginning of the study was 31.3 ± 2.3 g and, other than a 1-2 g decrease in the days following surgery, was not significantly different after 28 days (30.7 ± 2.4 g).

AngII Pump Implantation

AngII-induced AAAs were created using a previously described murine model.1 Briefly, osmotic pumps (model 2004, Durect Co., Cupertino, CA USA) were surgically implanted such that AngII (A9525, Sigma-Aldrich, St. Louis, MO USA), dissolved in Dulbecco’s phosphate buffered saline (DPBS, Invitrogen, Carlsbad, CA USA), was delivered subcutaneously at a rate of 1000 ng/kg/min (n=31). Of these, 16 survived to day 28 and 11 developed suprarenal AAAs defined as an expansion of at least 50% over the normal lumen diameter. The 5 mice that did not develop AAAs had no unusual aortic curvature or motion, suggesting the lack of aneurysms were due to variability in the model. Pumps were not primed prior to implantation, meaning a drug delivery start-up gradient was likely present for the first 40 hours. This method has been shown to typically produce AAAs directly above the right renal artery,2 usually between days 4 and 10 post-implantation.3 Our 28 day survival rate was 52%, with most mortality occurring between
days 3 and 10 due to aortic rupture, leading to hemorrhage in either the thoracic or abdominal
cavities. As a control, another cohort of mice was implanted with osmotic pumps filled with
DPBS (n=6). Buprenorphine at 0.05 mg/kg was given to each mouse in two 50 µl subcutaneous
injections before and after surgery (Bedford Labs, Bedford, OH USA).

Elastase Aneurysm Initiation

A detailed description of the intra-aortic elastase infusion has been previously
published.\textsuperscript{4,5} Briefly, the infrarenal aorta and bilateral iliac arteries were exposed via midline
laparotomy. All aortic branches within one centimeter of the trifurcation were ligated, and
temporary 6-0 silk ligatures were placed around the proximal and distal portions of the aorta. The
distal part of the aorta was cannulated with heat-tapered polyethylene tubing (PE-10; Baxter
to create a pressure head of 100 mmHg, the aorta was filled with saline containing 4.5 U/mL
Type I porcine pancreatic elastase for 5 minutes (n=28, specific activity 5 U/mg protein; E1250;
Sigma Chemical Co., St. Louis, Missouri, USA). The control group of mice underwent aortic
infusion with heat-inactivated elastase, in which the standard dose elastase solution was heated to
100°C for 20 minutes to eliminate enzymatic activity (n=6).\textsuperscript{5} The aorta typically dilates to 150-
170% of its original diameter during the elastase infusion. The infusion catheter was then
removed and the aortotomy closed with a 10-0 suture. The intestines were returned to the
abdomen and the wound was closed in two layers with 6-0 nylon and polypropylene sutures.
Buprenorphine at 0.05 mg/kg was given to each mouse in two 50 µl subcutaneous injections
before and after surgery (Bedford Labs, Bedford, OH USA). Of the animals undergoing this
procedure, 12 elastase-infused and 6 heat-inactivated elastase control mice survived to day 28,
with most mortality occurring within the first three days after surgery. All mice that survived to
day 28 and were given active elastase developed infrarenal AAAs, defined as at least a 50% dilation when compared to the normal lumen diameter proximal to the expansion.

**Magnetic Resonance Vessel Imaging**

The following MR imaging procedures have been described in more detail previously.\(^6\) All MR imaging was performed at 4.7 T using a Direct Drive console (Varian, Inc., Palo Alto, CA, USA) and a 4 cm inner diameter transmit-receive volume coil (Morris Instruments Inc., Ottawa, Ontario, Canada). All animals were imaged before surgery (day 0), and on days 3, 7, 14, 21, and 28. A 3D time-of-flight (TOF) sequence, which highlights flowing blood in the aorta, was used to acquire data above and below the renal arteries (repetition time (TR) 15 ms, echo time (TE) 3 ms, field-of-view (FOV) (3 cm)\(^3\), flip angle (\(\alpha\)) 20\(^\circ\), matrix 128\(^3\) zero-filled to 256\(^3\), slab thickness 15 mm, number of excitations (NEX) 2). A 4 mm saturation band was placed approximately 2 mm below the excitation slab to null the signal from venous flow. Coronal and sagittal maximum intensity projections (MIPs) of these images were used to plan image planes at levels between the celiac and right renal artery (suprarenal – SR; location of AngII-induced AAAs) and halfway between the left renal artery and aortic trifurcation formed by the two iliac and tail arteries (infrarenal – IR; location of elastase-induced AAAs). A cardiac-gated 2D TOF sequence with 12 cine frames was used to quantify lumen motion throughout the cardiac cycle (TR 96-126 ms depending upon heart rate, TE 4 ms, FOV (2 cm)\(^2\), \(\alpha\) 20\(^\circ\), matrix 128\(^2\) zero-filled to 256\(^2\), slice thickness 2 mm, NEX 8). A saturation band (thickness of 20 mm) was placed approximately 2 mm below the slice of interest to null the venous signal. Two subcutaneous ECG leads and a respiratory monitor (SA Instruments, Inc., NY) were utilized for prospective triggering off the R-wave only during exhalation. The total imaging time per animal was approximately 40 minutes.
Image Data Analysis

Aortic Centerline Shift

The magnitude and direction of AAA expansion was determined at the suprarenal location with the greatest shift between the aneurysm centerline and the centerline of an arbitrarily placed theoretical healthy aorta. These healthy vessels were produced by creating segmentations that followed the natural curvature of the vessels and were approximately the same size as the surrounding healthy aorta. The centroids of 100 segmentations along the aorta from the 3D TOF scans were calculated to create these centerlines. Irregularities along the centroid-based centerline were removed by Fourier smoothing. The magnitude of the shift is the actual displacement, and the angle is defined relative to anterior (0°), such that a positive angle corresponds to a leftward shift and a negative angle to a rightward shift.

Aortic Curvature

Methods for quantifying geometric curvature from vessel centerlines have been described previously. Briefly, three points placed along the centerline were used to define a circle (Figure 2A). The total distance between the three points, or window size, was set to approximately twice the aortic diameter (2 mm suprarenally, 1.5 mm infrarenally) at each image location. The geometric curvature (κ) was defined by the inverse of the radius of this circle as follows:

\[
\kappa = \frac{1}{\text{radius}} \text{ (mm}^{-1})
\]  

(S.1)

Curvature was calculated in increments of 0.1 mm down the length of the abdominal aorta before aneurysm formation. The location of the maximum curvature before aneurysm formation was calculated in reference to the right renal artery. Curvature analysis after AAA formation was not performed, as asymmetric vessel expansion created locations of increased centerline curvature at the proximal and distal sections of the aneurysm.
Aortic Lumen Centroid Motion

From the 2D TOF images, the lumen boundary in each frame was defined using thresholding, followed by manual correction (if necessary) depending on image quality, with vascular modeling software. These segmentations were then Fourier smoothed to eliminate high frequency noise (8 modes). As described previously, the lumen centroids were calculated from these segmentations using Matlab (R2008a, MathWorks, Natick, MA USA). The magnitude of centroid motion was determined by finding the largest distance between centroids at all individual timeframes and the initial timeframe, with the maximum magnitude usually occurring at peak systole. The direction of lumen centroid motion was determined at this timeframe by measuring the angle between anterior (defined as 0°) and the centroid motion vector. In other words, the centroid of the initial timeframe was defined as the origin and the magnitude and angle of centroid motion were calculated in reference to this location.

Correlation Factor

In order to compare the direction of aortic motion and AAA expansion, a correlation factor between two angles was defined as follows:

\[
\text{correlation factor} = \cos (\text{angle}1^\circ - \text{angle}2^\circ)
\]  

(S.2)

Thus, a difference between angles of 0° corresponds to a factor of one and shows total correlation, a difference of 90° corresponds to a factor of zero, and a difference of 180° corresponds to a factor of negative one (reflecting an opposite correlation).

Strain Quantification

Using the same segmentations as the Aortic Lumen Centroid Motion calculations discussed above, the perimeter (P) of each segmentation was calculated by summing the distances between consecutive points around the circumference (MATLAB, The Mathworks...
Maximum Green-Lagrange circumferential cyclic strain was defined as:

$$\varepsilon_{\theta\theta} = \frac{1}{2} \left[ \left( \frac{P_{\text{sys}}}{P_{\text{dias}}} \right)^2 - 1 \right] \times 100\% \tag{S.3}$$

where $P_{\text{sys}}$ and $P_{\text{dias}}$ are the perimeters of the vessel at systole and diastole, respectively. Strain waveforms, representing the in vivo deformation undergone by the vessel over the cardiac cycle, were created by calculating strain for every image, using $P_{\text{dias}}$ as the unloaded dimension. Each timeframe is one image of the 2D cardiac-gated MRA sequence and represents one time point during the cardiac cycle. Since the TR of this sequence was set depending on the period of the individual mouse’s heart rate, the timing across animals varied slightly but was approximately 10 ms for each frame. As an example, with a TR = 120 ms and 12 frames, we would acquire 12 images spaced approximately 10 ms apart throughout the cardiac cycle.

**Ultrasound**

For comparison, the same groups of mice were imaged at day 28 with a small animal US system (Vevo770; VisualSonics Inc., Toronto, Canada). The diminutive vessels and rapid heart rates in rodents required a transducer stabilizing system consisting of a heated platform to maintain body temperature and a transducer mount with an integrated rail system. A depilatory cream was used to remove hair over the abdomen before imaging. Images from an anteroposterior projection over the aorta were collected with a single element transducer (RMV-707B; center frequency, 30MHz; focal length, 12.7 mm; axial resolution, 55 µm; lateral resolution, 115 µm). M-mode tracings, from which systolic (maximum) and diastolic (minimum) diameters were measured for 6 or more cardiac cycles per mouse, were collected at the
maximum AAA diameter. Average Green-Lagrange strain was estimated from these cardiac cycles using diameters instead of perimeters in equation 3.

**Blood Pressure**

Blood pressure was non-invasively measured by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff at days 0, 3, 7, 14, 21, and 28 (CODA System, Kent Scientific, Torrington, CT).\(^1\) All mice were anesthetized at the same level of isoflurane as during *in vivo* imaging. Maximum cuff pressure was set to 250 mmHg, with 20 seconds for each inflation run. Systolic, mean, and diastolic pressures were collected from 7-18 animals per group at each time point. The control group for the blood pressure data is a combination of mice with saline pumps and others that did not undergo pump implantation. Pressure measurements were not collected from every animal at all time points due to some animals developing slight tail damage from heating and the occlusion cuff.

**Ex Vivo Endpoint Preparation**

Mice were sacrificed by asphyxiation with carbon dioxide. The heart was exposed and the aorta was pressure perfused with 4% paraformaldehyde (PFA, EM Grade, Electron Microscopy Sciences, Hatfield, PA USA) at approximately 100 mmHg of pressure for 5 minutes using a peristaltic pump (66, Harvard Apparatus, Holliston, MA USA). A low melting temperature agarose (1% by weight) was then injected and allowed to solidify to help keep the aortic lumen expanded during dissection (SeaPlaque® GTG® Agarose, Lonza, Rockland, ME USA). The entire aorta from the arch to the iliac arteries was harvested and digital pictures were taken of both dorsal and ventral surfaces (D100 with a Nikkor 105 mm lens, Nikon Inc., Melville, NY USA). Maximum aneurysm diameter was measured from these images.

**Histology**
All aortas were striped with a small paint brush and colored dyes for circumferential orientation (blue for anterior, green for left, yellow for posterior, red for right), then processed and embedded in paraffin. Axial sections 5 μm in thickness were stained with hematoxylin and eosin (H&E) and elastic-Van Gieson (EVG 87017, Richard-Allen Scientific, Kalamazoo, MI USA) to visualize elastic lamina. Other sections were stained for smooth muscle cells using an anti-smooth muscle actin antibody (NB600-531, Novus Biologicals, Littleton, CO USA) and macrophages using an F4/80 antibody (MCA497GA, AbD Serotec, Raleigh, NC). Axial cross-sections at locations separated by 150-300 μm were taken and scanned with a NanoZoomer Digital Pathology System (Olympus, Melville, NY USA). Histological sections were qualitatively compared to \textit{in vivo} MR images at comparable axial locations and extent of elastin breakdown was recorded. In the AngII model, the circumferential location of adventitial hematoma and the octant corresponding to the middle of the medial elastin rupture were recorded (anterior, anterior-left, left, etc.). With the elastase-induced AAAs, the amount of medial elastin area was quantified by drawing a region of interest (ROI) in both the anterior and posterior regions, followed by a thresholding technique that produced a binary image of only the elastin pixels (MATLAB). In addition to elastin area, the inner vessel perimeter was calculated for both regions. Elastin area was then divided by the inner vessel perimeter to produce a normalized medial elastin area metric that was independent of the size of the ROI.

\textbf{Near-Infrared Fluorescence}

Two activatable fluorescent probes allowed for imaging of the spatial distribution of relative protease activity associated with AAA disease (MMPSense™ 680 and ProSense® 750, VisEn Medical, Bedford, MA USA). MMPSense™ 680 is activated by matrix metalloproteases (MMP)-1, -2, -7, -9, -12, and 13, while ProSense® 750 is activated by cathepsins B, D, G, K, L,
and S. Probes were administered through a tail or jugular vein injection 24 hours before sacrifice at day 28 (180 µl MMPSense™ 680, 18 µl ProSense® 750). Fluorescent images of the excised aortas from both ventral and dorsal surfaces were acquired at 650 nm excitation/700 nm emission (MMPSense™ 680) and 730 nm excitation/790 nm emission (ProSense® 750) with the following parameters: 60 s exposure, (50 mm)² FOV, 0 binning (FX Pro, Kodak Molecular Imaging Systems, New Haven, CT USA). These images were then converted to tiffs using Matlab (R2006b) and mean signal intensities in regions-of-interest were measured using ImageJ (version 1.6.0_05, NIH, USA). Mean signal intensity was measured in the AAA (I_{AAA}), the normal thoracic aorta (I_{thoracic aorta}), and the background noise (I_{noise}). A ratio of signal intensity was used to estimate the relative fold change as:

$$\frac{I_{AAA} - I_{noise}}{I_{thoracic aorta} - I_{noise}}$$

(S.4)

Each AAA was then divided into four approximately equal sized quadrants of the aneurysm (I_{quadrant} defined clockwise as: RS – right superior, LS – left superior, LI – left inferior, and RI – right inferior). A signal intensity ratio for each quadrant, normalized to total AAA signal, was used to determine probe accumulation patterns within each AAA and defined as the following:

$$\frac{I_{quadrant} - I_{noise}}{I_{AAA} - I_{noise}}$$

(S.5)

**Statistical Analysis**

All data are reported as mean±standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA) using JMP (SAS Institute, Inc., Cary, NC USA). Within each ANOVA, the P-values used to assess the statistical significance of pairwise comparisons were adjusted for multiplicity by the Tukey–Kramer method.
REFERENCES


SUPPLEMENTAL FIGURE LEGENDS

Figure S.I. Vascular models and corresponding MIPs for 10 AngII-induced AAAs at day 28.

Figure S.II. Vascular models and corresponding MIPs for 10 elastase-induced AAAs at day 28. The right testicular artery is typically shifted rightward and is highlighted (arrow). This suggests reduced support in the tissue along the right side of the aorta as a result of the surgical procedure.

Figure S.III. A) Curvature, defined as radius\(^{-1}\), was calculated before aneurysm formation (the green line is the aortic centerline). B) Overlapping coronal MR MIPs of a healthy vessel from the distal thoracic aorta to the iliac arteries. The location of maximum curvature at day 0 was 2.43±0.54 mm above the right renal artery (arrow) and was directed leftward (91.5±15.0°).

Figure S.IV. Mice given AngII showed a significant increase in systolic, diastolic, and pulse pressures compared to day 0 (*p<0.05).

Figure S.V. Comparison of serial histology and MR images of an AngII-induced AAA. Coronal and sagittal MIPs (A) show typical leftward vessel expansion (white bracket). A total of seven locations (each separated by 260-280 µm) were stained with elastic-Van Gieson (EVG) for visualization of elastin. Similar locations are shown for the MR images, highlighting true and false lumens (asterisks in first five sections), with a large organizing hematoma (sections 6 and 7). Segmentations included the flow lumen and adventitial hematoma, such that the vessel boundary was approximately circular. Black bars represent 1 mm on the histological images, and MR voxel resolution was (117 µm)\(^3\).

**Figure S.VI.** Example immunohistochemistry AngII AAA sections. Part A shows 50x magnification examples of three different suprarenal aneurysms (black bars 1mm). While the size and amount of thrombus differ, all three have focal breakages in the medial elastin. This is highlighted in both the hematoxylin and eosin (H&E; B) and elastic-Van Gieson (EVG; C) stains with arrows (200x magnification). The arrowheads highlight smooth muscle cells with a SMA stain (D) and macrophages with an F4/80 stain (E; black bars 0.1mm).

**Figure S.VII.** Comparison of serial histology to MR images of an elastase-induced AAA. The aneurysm shown here is long and tortuous aneurysm (A). This vessel was sectioned at eight locations, each separated by 160-200 µm (B). Orientation is labeled as S – superior, I – Inferior, A – anterior, P – posterior, L – left, and R – right. Black bars represent 1 mm on the histological images, and MR voxel resolution was $(117 \, \mu m)^3$.

**Figure S.VIII.** Example histological AAA images of elastase AAAs at 200x. Intimal thickening is seen in the hematoxylin and eosin (H&E; A), while the elastic-Van Gieson (EVG; B) shows complete degradation of the medial lamellar units within this region. Significant SMC apoptosis is seen with the SMA stain (arrows, C), while infiltration of macrophages into the media and neointima can be seen in D (arrowheads). A heat-inactivated elastase control vessel is shown in images E-H. These show intact MLUs and less inflammation within the vessel wall. Black bars represent 0.1 mm.
**Figure S.IX.** The left inferior quadrant for images of both matrix metalloproteinases (MMPs) and cathepsin probes in an AngII AAA (L – left, R – right; S – superior, I – inferior; A) have significantly increased accumulation of signal compared to all other quadrants (*p<0.01; B).
SUPPLEMENTAL TABLES

Table S.I. Magnitude and direction of abdominal aortic curvature before AAA formation. The suprarenal group went on to develop AngII AAAs, while those in the infrarenal group underwent laparotomies and developed elastase-induced aneurysms. The amount of vessel curvature was significantly less in the infrarenal aorta when compared to the suprarenal region (*p<0.05).

<table>
<thead>
<tr>
<th>Aortic location</th>
<th>n</th>
<th>Geometric curvature (mm⁻¹)</th>
<th>Direction of curvature (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suprarenal</td>
<td>11</td>
<td>0.16 ± 0.02</td>
<td>91.5 ± 15.0</td>
</tr>
<tr>
<td>Infrarenal</td>
<td>12</td>
<td>0.09 ± 0.02*</td>
<td>-14.5 ± 44.6</td>
</tr>
</tbody>
</table>
Table S.II. Correlation factors between the direction of aneurysm expansion at day 28, centroid motion at day 0, and aortic curvature at day 0. For the AngII group, expansion, motion, and curvature were measured in the suprarenal aorta, while the elastase group had these metrics measured in the infrarenal aorta. A correlation close to one is seen in the AngII model for all three factors. The elastase model shows little correlation between AAA expansion and vessel curvature or motion.

<table>
<thead>
<tr>
<th>Correlation Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine AAA Model</td>
</tr>
<tr>
<td>AngII</td>
</tr>
<tr>
<td>Elastase</td>
</tr>
</tbody>
</table>
Table S.III. The locations of medial elastin breakage and adventitial hematoma formation were qualitatively determined by delineating 8 regions around the circumference of each AngII AAA. In cases where the medial elastin broke and then spread apart, the location midway between either side of the breakage was determined. Medial breakage in one AAA could not be located, likely due to sectioning proximal or distal to the location of medial lamellar unit breakdown. Most of the elastin breakages occurred in the left anterior direction, whereas most of the hematomas formed to the left. In all cases, the right and posterior sides of these AAAs looked normal, with no elastin breakage or hematoma formation.

<table>
<thead>
<tr>
<th>Circumferential Region</th>
<th>anterior</th>
<th>left-anterior</th>
<th>left</th>
<th>left-posterior</th>
<th>posterior</th>
<th>right-posterior</th>
<th>right</th>
<th>right-anterior</th>
<th>unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of medial elastin breakage</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Number of AAAs</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Location of adventitial hematoma</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure S.I

Day 28
Figure S.III

A) Suprarenal Curvature

B) Coronal Overlapping MIPs
Figure S.IV

**Systolic Blood Pressure**

- AngII: Red squares
- Control: Blue circles

- *p < 0.05*

**Diastolic Blood Pressure**

- AngII: Red squares
- Control: Blue circles

- *p < 0.05*

**Pulse Pressure**

- AngII: Red squares
- Control: Blue circles

- *p < 0.05*
Figure S.V

A) AngII AAA 3D MIPs
   - Coronal
   - Sagittal

B) EVG Histology MRI Slices
   - Proximal
     1)
     2)
     3)
     4)
     5)
     6)
     7)

   - Distal
Figure S.VII

A) Elastase AAA 3D MIPs
   Coronal  Sagittal

B) EVG Histology  MRI Slices
   Proximal

1)  
2)  
3)  
4)  
5)  
6)  
7)  
8)  
Distal
Figure S.IX

A) Suprarenal AAA Quadrants

B) Normalized AngII AAA NIRF Signal Intensity

<table>
<thead>
<tr>
<th>Quadrants</th>
<th>MMPSenseTM680 Ventral</th>
<th>MMPSenseTM680 Dorsal</th>
<th>ProSense®750 Ventral</th>
<th>ProSense®750 Dorsal</th>
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</thead>
<tbody>
<tr>
<td>RS</td>
<td>0.83</td>
<td>1.25</td>
<td>1.28</td>
<td>1.04</td>
</tr>
<tr>
<td>LS</td>
<td>0.77</td>
<td>1.38</td>
<td>1.40</td>
<td>1.02</td>
</tr>
<tr>
<td>LI</td>
<td>0.91</td>
<td>1.28</td>
<td>1.38</td>
<td>1.00</td>
</tr>
<tr>
<td>RI</td>
<td>0.91</td>
<td>1.38</td>
<td>1.40</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* *p < 0.01